Universidade de Lisboa

Faculdade de Farmácia

Functionalized Drug Delivery Systems for Cancer Treatment

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Abstract

Cancer is now a worldwide problem, responsible for a high rate of morbidity and mortality. For many years, the primary used therapies to treat cancer were chirurgical removal of the cancer, radiation therapy and chemotherapy. Although lots of times these types of therapies can bring good results, there is still many flaws when it comes both to eradicating the tumor cells completely and potential side effects either from radiation or chemical cytotoxicity. In an effort to overcome these obstacles, many new strategies have been developed to target cancer cells specifically, leaving the healthy tissue and cells unharmed, and also to improve circulation time, drug retention in tissues and general efficacy. These targeted drug delivery systems can be divided into Vascular Disruptive Agents and Angiogenesis Inhibitors which function by blocking the tumor vasculature and preventing neovascularization, consequently blocking the cancer source of nutrients and growth. Therefore, the most recent strategies are based in drug loaded nanoparticles, that can passively or actively target cancer cells through specific ligands and then release its content into the cancer tissue and cells. Investigation of new receptor types and their respective ligands expressed in tumor cells is essential for new techniques that can surpass current restrictions, clearance effect and tumor specificity without damaging the healthy cells and tissues.

In this master thesis it is possible to understand what the current most used agents, ligands, and carriers in functionalized drug delivery systems and how new strategies are being studied every single day to better target and better perform in the eradication of cancer cells.

Keywords: Cancer, Functionalized drug delivery systems, Targeting, Nanocarriers, Ligands

Resumo

O cancro é, hoje em dia, um problema a nível mundial, responsável por uma elevada taxa de morbilidade e mortalidade. Desde há muitos anos que as terapias mais utilizadas para tratar o cancro passam pela remoção cirúrgica do tumor, pela radioterapia e a quimioterapia. Embora estes tipos de terapias possam trazer bons resultados e melhorias, ainda existem muitas falhas tanto na erradicação completa de células tumorais como possíveis efeitos colaterais causados durante e após o tratamento .

Com vista a superar estes obstáculos, muitas novas estratégias foram desenvolvidas para atingir especificamente células tumorais, deixando tecidos e células saudáveis sem danos, além de procurarem também melhorar o tempo de circulação, retenção nos tecidos-alvo e eficácia geral. Estes sistemas funcionalizados podem ser divididos em agentes "disruptivos vasculares" e inibidores da angiogénese, e funcionam bloqueando a vasculatura do tumor e impedindo a neovascularização, bloqueando a fonte de nutrientes e, consequentemente, o crescimento do cancro. Por sua vez, as estratégias mais recentes envolvem também nanopartículas que encapsulam fármacos no seu interior, que atingem de maneira passiva ou ativa as células tumorais e libertam o seu conteúdo no local alvo. A investigação de novos de novos tipos de recetores e respetivos ligandos expressados em células tumorais é preponderante para que novas estratégias possam ultrapassar os obstáculos correntes.

Nesta dissertação de mestrado serão descritos os agentes, ligandos e meios de transporte atuais mais utilizados nos sistemas de veiculação funcionalizados e serão exemplificadas algumas dessas estratégias estão a ser estudadas todos os dias para melhorar o direcionamento específico e para melhorar os resultados na erradicação das células tumorais.

Palavras-chave: Cancro, Sistemas de veiculação funcionalizados, Direcionamento, Sistemas de veiculação, Ligandos

Abbreviations

AR-CPP - Active tumor-targeting arginine-rich cell penetrating peptides
bFGF - Basic fibroblast growth factor
CA4P - Combretastatin A-4 disodium phosphate
CSNPs - Core-shell structured nanoparticles
DOX – Doxorubicin
EPR- Enhanced permeability and retention
EGFR - Epidermal growth factor receptor
FGF - Fibroblast growth factor
FR- Folate receptor
MDR- Multi drug resistance
MPS/RES - Mononuclear phagocyte system / Reticuloendothelial system
MTDA- Micro Tubule – Destabilizing Agents
PDGF- Platelet derived growth factor
PEG- Polyethylene glycol
PLA- Polylactic acid
PLGA- Poly(D,L-lactic-co-glycolic acid**)**
PSMA- Prostate specific membrane antigen
PVA-Polyvinyl alcohol
SELEX- Systematic evolution of ligands by exponential amplification
TfR- Transferrin Receptor
VDA- Vascular disruptive agent
VEGFR - Vascular endothelial growth factor receptor
VEGF - Vascular endothelial growth factor
WHO – World Health Organization

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This review aims to present an overview of the current knowledge and recent developments regarding targeting therapies during the last three decades (late 1990s to early 2010s) as well as to explore potentially therapeutic options for the near future. A search of MEDLINE was conducted using the term "cancer" in combination with term "ligands", "targeting methods", "novel therapies". References from the most representative reviews (in author's personal opinion) and clinical study publications and abstracts of scientific congresses were searched.

1. Introduction

1.1. Cancer nowadays

Cancer is considered to be the greatest barrier in increasing the life expectancy in every country of the globe, being the leading cause of death worldwide. As reported by the World Health Organization (WHO), the first and second leading cause of death before age 70 in 53% of the countries is cancer (1). Also, WHO predicts an increase of approximately 70% of new cancer cases, in the next 20 years (2).

The reasons why cancer incidence and mortality are quickly rising globally are complex, as they reflect both population growth and aging, plus an evolution of the distribution and prevalence of the main cancer risk factors. Exposure to infectious agents and hormones, radiation and environmental occupational are alarming risks of cancer associated with socioeconomic development and it can be seen when comparing Figures 1 and 2 (1,2).

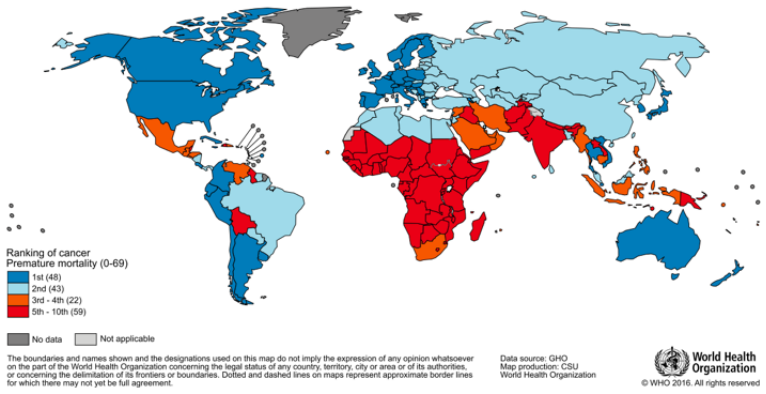

Figure 1. Global Map Presenting the National Ranking of Cancer as Cause of Death at Ages Below 70 Years. Source: World Health Organization (1).

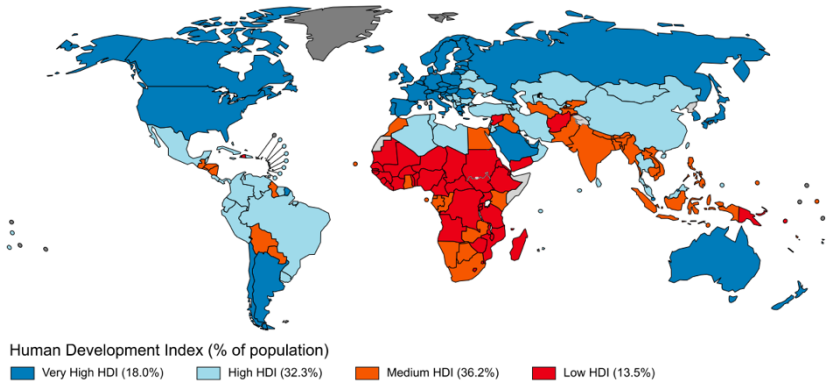

Figure 2. Global Map Presenting a 4 tier Human Development Index. Source: United Nations Procurement Division/United Nations Development Program (1)

Approximately between one third and one half of all cancers could be avoided by adopting a healthier lifestyle, cutting out the tobacco use, moderating alcohol consumption, embracing an active lifestyle and maintaining a healthy body mass index (2).

Cancer survivorship is a progressively important public health concern, as cancer patients are quickly growing and face risks of recurrence and long-term adverse treatment related effects, added to high risks of physical and psychosocial related comorbidities. Nevertheless, the diagnosis of cancer can be viewed as new start, since patients are more motivated to adjust to a healthier lifestyle and behaviors (3).

1.2. Statistics

According to the Table 1 and Table 2 adapted from Global Cancer Statistics 2018 by the Global Cancer Observatory (GLOBOCAN), in 2018, there were estimated 18.1 million new cases and 9.6 million cancer deaths.

When comparing the distribution of all-cancer incidence and mortality according to world area for both genders combined and then for men and women separately, we can conclude that: nearly one half of the new incidents and more than one-half of cancer deaths will occur in Asia. Twenty-three percent (24.4%) of the total cancer cases and 20.3% of the cancer deaths would occur in Europe, despite it representing hardly 9% of the world population (Figure 3) (1,4).

Globally, the incidence rates vary across all the regions: regarding males, when comparing the incidence rate between Australia/ New Zealand and Western Africa, there is a 6-fold variation, from 571.2 per 100,000 to 95.6 per 100,000

Table 1. Top 10 Cancers with highest number of new cases and deaths in the year 2018 (1,4)

CANCER SITE	N.º OF NEW CASES (% OF ALL SITES)	CANCER SITE	N.º OF DEATHS (% OF ALL SITES)
Lung	2,093,876 (11.6)	Lung	1,761,007 (18.4)
Breast	2,088,849 (11.6)	Stomach	782,685 (8.2)
Prostate	1,276,106 (7.1)	Liver	781,631 (8.2)
Colon	1,096,601 (6.1)	Breast	626,679 (6.6)
Nonmelanoma of skin	1,042,056 (5.8)	Colon	551,269 (5.8)
Stomach	1,033,701 (5.7)	Esophagus	508,585 (5.3)
Liver	841,080 (4.7)	Pancreas	432,242 (4.5)
Rectum	704,376 (3.9)	Prostate	358,989 (3.8)
Esophagus	572,034 (3.2)	Cervix uteri	311,365 (3.3)
Cervix uteri	569,847 (3.2)	Rectum	310,394 (3.2)
All sites	**18,078,957**	**All sites**	**9,555,027**

respectively. Regarding females, there was a nearly 4-fold variation when comparing Australia/ New Zealand to 96.2 per 100,000 in South-Central Asia. Furthermore, it is estimated that the incidence rate for all cancers combined is approximately 20% larger in men (218.9 per 100000) than in women (182.6 per 100000) (1,4).

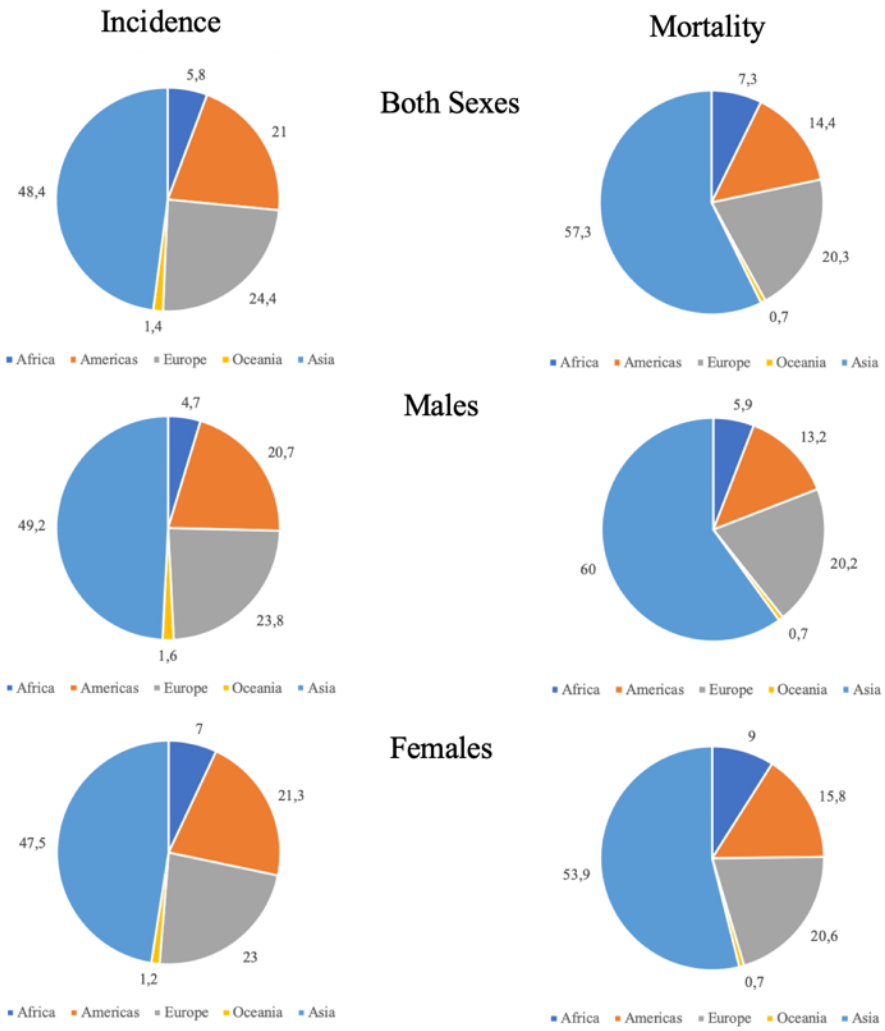

Figure 3 Estimated percentage of incidence and mortality of cancer by continent, in males, females and both sexes (1,4)

It can be noticed that these variations are a reflection of the difference of factors as type of exposures, possible associated cancers, and mostly by the accessibility and use of diagnostic and screening services. This explains why the greatest incidence rate in both genders are found in Australia/ New Zealand (1). It results from a boost of skin cancer detection, particularly non-melanoma skin cancer, associated with a higher risk.

Following the incidence trend, the death rate is 50% higher in men than in women, for all cancers combined worldwide.

Death rates vary across the regions, from 171.0 per 100,000 in Eastern Europe, to 67.4 per 100,000 in Central America regarding man, and from 120.7 in Melanesia to 64.2 in Central America and Eastern Asia concerning woman. Surprisingly, it is estimated that

the risk of a women dying from cancer in East Africa is higher than in Northern Europe, Australia/ New Zealand and North America (1,4).

Table 2. Number of new cases and deaths of 17 other cancers in 2018 (1,4).

CANCER SITE	N.º OF NEW CASES (%OF ALL SITES)	N.º OF DEATHS (% OF ALL SITES)
Thyroid	567,233 (3.1)	41,071 (0.4)
Bladder	549,393 (3.0)	199,922 (2.1)
Non-Hodgkin lymphoma	509,590 (2.8)	248,724 (2.6)
Pancreas	458,918 (2.5)	432,242 (4.5)
Leukemia	437,033 (2.4)	309,006 (3.2)
Kidney	403,262 (2.2)	175,098 (1.8)
Corpus uteri	382,069 (2.1)	89,929 (0.9)
Lip, oral cavity	354,864 (2.0)	177,384 (1.9)
Brain, nervous system	296,851 (1.6)	241,037 (2.5)
Ovary	295,414 (1.6)	184,799 (1.9)
Melanoma of skin	287,723 (1.6)	60,712 (0.6)
Gallbladder	219,420 (1.2)	165,087 (1.7)
Larynx	177,422 (1.0)	94,771 (1.0)
Multiple myeloma	159,985 (0.9)	106,105 (1.1)
Nasopharynx	129,079 (0.7)	72,987 (0.8)
Oropharynx	92,887 (0.5)	51,005 (0.5)
Hypopharynx	80,608 (0.4)	34,984 (0.4)
All sites	**18,078,957**	**9,555,027**

According to Global Cancer Statistics 2018, considering both genders before age 75 years globally, there is a 21.4% risk of developing cancer and 17.7% risk of dying from it. In general, 1 in 5 men will develop the disease, and 1 in 8 will die from it, while regarding women, 1 in 8 will develop it and 1 in 10 will die from it (1,4).

2. Conventional Therapies of Cancer

Cancer treatment has evolved greatly over time, with very significant improvements for the patient, concerning efficacy and quality of life.

When comparing the therapies and technologies of today with past ones, nowadays they are less invasive and toxic, allowing to reduce their side effects so that the patients can live their lives more naturally (1,5).

Conventional therapies generally consist in radiation therapy, cancer surgery, and drug administration such as, chemotherapy and hormone therapy. The use of these three different treatments can be done individually or in synergy, depending on factors as: the type of the cancer, staging of the disease, general health condition of the patient, the individual choices of each patient, and other associated conditions.

Chemotherapy and hormone therapy are treatments that use one or more anti-cancer drugs generally into the bloodstream to destroy cancer cells throughout the body. It can be either intended to cure or to reduce symptoms and prolong life.

Radiotherapy and surgery are used in local therapy, meaning the treatment is performed in a specific area of the body, as opposed to the drug administration treatment. In cancer surgery, the objective is to remove the tumor and nearby cancer tissue in the hope to eradicate the tumor. In radiotherapy, large doses of radiation are used to suppress cancer cells and reduce the tumor.

2.1. Surgery

Surgery is without a doubt the main procedure for the predominating part of cancers cases (6). The main objective of the oncological surgery is to remove the cancer physically, as it is generally known that in early stage of the disease, this type of treatment leads to long remissions (6). It is a common mistake to the general public to believe that the success of the treatment relies completely on the skills of the surgeon that conducts the procedure. The idea of the possibility of removing the tumor completely is based on the theory that the tumor is a local disease confined to a determined organ. It has been studied and confirmed that the tumor is not a respective organ disease, but a complex interrelation between the tumor and the host (6).

Thinking radically, in the earliest stage of the cancer, with no metastases in the organs, it could be possible to have a complete removal of the tumor. However, there is a

considerable amount of challenges when thinking practically: There is still a big gap between the beginning of the disease and the moment of the diagnosis, as 60% is discovered in the advanced stage, when surgery is no longer possible (7).
Furthermore, even in the event of an early diagnosis, there is the possibility of micro metastases or groups of single tumor cells not being detected by the most commonly used diagnostics methods, and these could be the trigger to a future recurrence of the disease. It is acknowledged that this type of procedure is associated with risks as dissemination of the cancer cells in to the blood stream, infections, immune system suppression and anesthesia related complications (8,9).
According to a study from the Institute of Experimental Pathology, Oncology and Radiobiology in Kiev, scientists were able to identify the presence of micro metastases in both the bone narrow and blood stream (33.1% and 28.3% respectively) in patients that were diagnosed without metastases (10).

2.2. Radiotherapy

Radiotherapy is considered to be an efficient and accessible method (6). It is often used in association with other types of surgery in the treatment of solid tumors. It can also be used as a palliative treatment with the objective of reducing the symptoms.
In general, radiotherapy acts by damaging the genetic material of the tumor, preventing it from growing and proliferate. On the other hand, there is still a great barrier in the way to successfully use this treatment, as the ionized radiation also affects the surrounding healthy tissues, which can aggravate the disease and develop secondary tumors. This can be seen in a study in the Lancet, that shows an increased mortality of 21% in lung cancer patients treated with both surgery and radiation, when compared to the group treated solely with surgery (6). Generally, these secondary tumors manifest after 5 to 9 years in leukemia and 10 or more years in solid tumors. Evidently, the risk of developing secondary tumors are dependent on factors such as, age, body part and radiation dosage (11).

2.3. Chemotherapy

Chemotherapy is the administration of chemical anticancer drugs usually administrated intravenously (12). The drug needs to pass through the vascular endothelium and occupy the interstitial and extravascular space (13). Simple diffusion according to the concentration gradient is the most common mechanism, in association with an increased vascular permeability (12,13). The greatest challenge here is to direct the chemotherapy concentration to the damaged cells without damaging the surrounding healthy cells.

The dose of drug that can reach to the target site is also a challenge. The relative mass of the organ and the total body mass is needed to determine the effective concentration of the drug which is attacking the tumor (14,15).

Combining drug concentration and time restrains, allows tumors to suffer mutation and to develop systems to reduce the chemotherapy action, in a way that can make the cancer more resistant and increase its viability (15). Early treatment cessation and premature death may occur, being consequence of chemotherapy side effects. Also, the natural immunological response can be harmed by the intravenous route because of the bone marrow suppression and lympholysis. In addition, metabolic and excretory processes including plasma binding and dynamic tumor microenvironment are other types of obstacles that can influence the intravenous route (15,16).

In general chemotherapy is limited by drug efficacy by itself but also multidrug resistance (MDR). These limitations are often due to the development of genetic mechanisms that overlap apoptosis even with all the cell DNA damaged caused by the chemotherapy. Furthermore, the number of side effects in surviving patients is alarming, making the scientific community focus more in a combination therapy instead of a single drug for each type of cancer. Thus, most effort that had been put in the study of a single drug has been redirected to overpower MDR and finding alternative routes that can integrate the therapeutic induction of apoptosis (17).

3. Targeted delivery of drugs

If we go back about 30 years ago, cytotoxic drugs were the main method in cancer chemotherapy. Although it would rapidly kill, it could not discriminate between healthy and cancer cells. In 1964, it was created a program called "Special Virus Cancer Program" to discriminate these cells. However, this program did not have that much success. Twenty years later, it was restarted but this time it discovered and identified a variety of oncogenes, tumor suppressors and signaling pathways associated with metastasis and oncogenic conversion (18). After this discovery, we could better understand the cell signaling transduction pathways, identify a variety of drug targets and sequencing the human genome. All this led to a mindset change in terms of cancer therapy study, as it became more driven towards a specific target (18).

It is known that the vascular system is the best candidate when it comes to targeted anticancer therapies. It is essential to tumor growth and metastasis, as it is part of the tumor microenvironment and responsible for nutrition and toxic waste removal (19).

The tumor vasculature is composed of endothelial cells that are genetically stable and less adaptive than tumor cells, which means there is a less probability of acquiring drug resistance. (20) However, K. Hida and M. Klagsburn said that some endothelial cells in solid tumors are aneuploid and genetically unstable (21). According to some studies, it is estimated that nearly 100 tumor cells are nurtured from one single endothelial cell, therefore, it could be more beneficial to design a therapy that targets the vasculature instead of the tumor, as it could achieve a greater anticancer effect (22,23).

All the vast variety of therapies can be divided into, vascular disrupting agents (VDAs) and angiogenesis inhibitors. The first one's target and blocks the existing tumor vasculature, while the second ones restrain neovascularization of the tumor. Together they have the goal to achieve tumor regression by devascularization of the tumor, which means, blocking the tumor source of nutrients and growth (19).

3.1. Vascular Disrupting Agents (VDAs)

It is known for many years that it is possible to induce cell death through hemorrhagic necrosis and ischemia by obstructing the blood supply to tissue. This was described for the first time in 1852 in testicular torsion when it was observed that twisting a spermatic cord would reduce the blood flow into the testicle, causing necrosis (18). Although it was

only in 1923 that William Woglam presented the potential vascular interruption to induce tumor death, describing how blocking the blood supply to cancer cells could lead to cancer regression and proposed new therapies in this matter. However he also described difficulties with the treatment, being the most preoccupant one effective targeting, meaning that the therapy should be targeted for the vessels adjacent to tumors and not to other ones (24). Only 60 years later this concept was actively investigated by Denekamp and colleagues, where they observed that using clamps to obstruct blood flow to transplanted cancers in mice caused cancer cell death proportional to the clamping time (25). To this day, methods have been created to identify the differences between normal and cancerous tissue. Being its main objective to have specificity to the tumor tissue and obstructing the tumor vessels leaving the healthy ones unaffected. It is possible to split these specific disrupting agents in to two different types, small molecule and ligand based. While small molecules take advantage of the physiological distinctions between normal and tumor vasculature do disrupt the vessels, ligand based VDAs uses ligands (antibodies, growth factors, peptides, etc…) that specifically bind to tumor receptors and then delivers agents able to destroy the cancer vessels (18).

3.1.1. Small Molecule VDAs

3.1.1.1 Microtubule-destabilizing agents

Since the tumor endothelium is immature and exceptionally proliferative, it depends in a tubulin cytoskeleton responsible for maintaining cell shape (25). Non tumor endothelium as more mature vasculature and has a much more settled actin cytoskeleton being much less reliant on the tubulin cytoskeleton when it comes to cellular functions (24,26).
Microtubule-destabilizing agents' function both as antimitotic and anti-vascular agents. As they destroy the tubulin cytoskeleton, they inhibit the spindle formation, the mitosis is interrupted in cancer cells and then the cancer vasculature crashes decreasing the blood supply (27). The main route of this drugs is to cause mitotic arrest with anti-vascular activity seen close to the maximum tolerated dose (18).

3.1.1.2 Combretastatin A-4 Disodium Phosphate

Combretastatin A-4 disodium phosphate (CA4P) is a microtubule-destabilizing agent that has anti-vascular effects below the maximum tolerated dose (28). CA4P binds to tubulin avoiding its polymerization as it is cleaved by endogenous phosphatases. It has been studied that within one hour of treatment, CA4P caused large vascular damage inducing hemorrhagic necrosis and consequent cancer growth delay (28,29). CA4P is currently being studied has potential therapeutic agent in non-small cell cancer, thyroid cancer and gynecological cancer. Patients treated with CA4P in combination with chemotherapeutics in a phase II/III clinical trial for advanced anaplastic thyroid cancer showed some positive results, as 26% of patients treated with CA4P and chemotherapy survived one year compared to only 9% in the patients not treated with CA4P (30).

3.1.1.3. Combretastatin Derivatives

After the good results of CA4P, combretastatin derivates were developed. A prodrug type of combretastatin A-1 has shown to be very efficient in the treatment of solid cancers such metastatic colorectal carcinomas and has been disclosed as having stronger anti-vascular and anti-cancer effects when compared to CA4P (31,32)

Ombrabulin (AVE8062) is other combretastatin derivative which has shown ability to cause large cancer core necrosis by quickly shutting down the cancer blood flow (33). In a different study in mouse models of squamous cell carcinoma, ombrabulin shown an increase of the anti-tumor activity of the treatment with radiation and cisplatin or cetuximab (34). Ombrabulin is in phase II trials combined with taxane and platinum drugs for the treatment of metastatic no-small cell lung cancer, in phase III for the treatment of soft tissue sarcoma, and in other phase I trials combined with other drugs in solid cancers (18).

3.1.1.4. N-Cadherin Antagonist

The N-Cadherin is a surface protein that is mediates the adhesive interactions between endothelial cells responsible for the functionality of the vasculature. ADH-1 is a cyclic peptide that inhibits the N-cadherin binding site and consequently decrease blood supply, causing hemorrhage necrosis in animal cancer models (35,36). ADH-1 has shown being well tolerated, showing some anti-cancer efficiency and is currently studied in phase I

trials combined with chemotherapy, and phase I and II trials in monotherapy in a variety of cancers (37).

3.1.1.5. Toxicity of VDAs

The most important problem that limits the development and use of small-molecule VDAs is its toxicity. ZD6126 is a drug that interferes with the tubulin cytoskeleton of endothelial cells and causes cell detachment (18). It has been demonstrated that this drug is able to cause a decrease of cancer blood flow and large endothelial cell loss (38), as well as able to cause extensive cancer necrosis in xenograft animal models (39). However, when ZD6126 proceeded to the clinical trials, it was shown that it had serious side effects at the therapeutically needed dose. Cardiotoxicity led to the interruption of a phase II clinical trial for the treatment of metastatic colorectal cancer and metastatic cell carcinoma. Following this tendency, nearly half of the small-molecule VDAs that progressed to clinical trials had their studies interrupted due to lack of efficiency when compared to its side effects (40). Their side-effects include myocardial infarction and cardiac ischemia, being accordant to their anti-vascular activity. It can be concluded that this side effects are a result of an insufficient specificity of small-molecule VDAs to tumor cells, meaning that they are arming healthy vasculature and cells (41). In experimental models, its being used the incorporation of ligands specific for cancer endothelium to improve the targeting of the molecules (42) as well as anti-hypertensive therapy as an effort of muffling their side effects (40).

3.1.2. Ligand-Directed VDAs

This class of VDAs has the ability to operate straight on the vasculature. Ligand-directed VDAs are composed of two molecules, first a ligand that binds with high specificity to the cancer vasculature and an effector, that once delivered to the cancer vasculature, damages it. Ligands can be, peptides, antibodies or growth factors (18). The effectors are bioactive molecules that can range from apoptosis inducing agents, cytotoxic agents and radioisotopes, to coagulation-inducing proteins, cytokines and toxins (18).

The first demonstration that this approach could be effective in the destruction of the cancer vasculature was in 1993, in a study where subcutaneous neuroblastoma tumors were set up in mice (43). The authors observed that the conjugated molecule caused a

quick reduction of the tumor while leaving the vasculature of healthy tissue unarmed(43). To continue to investigate this treatment, its imperious that the search for new targets on the cancer vasculature for ligands to bind to continues. Over the past years, a great amount of cell surface molecules has been discovered being regulated on the cancer vasculature. This search for more cell surface targets is a must in this therapy strategy (18).

3.2. Angiogenesis Inhibitors

Tumor cells in general originate from a single cell that as suffered numerous genetic events, making it possible for the cell to surpass the normal growth control mechanisms. In an early stage, the cancer cell can grow and develop into a tumor getting sufficient oxygen and nutrients by diffusion from normal vessels. Yet, as the tumors mass increases, it reaches a point that the normal vessels are no longer able to nurturer and oxygenate every single cancer cell. When this happens, the tumors growth becomes more restricted and localized, and to keep developing and metastasizing it must develop its own blood supply (19).This vascularization of a tumor is called Angiogenesis. This is a complex and multistep process, created by the combination hypoxia and cellular transformation (44).
The secretion of pro-angiogenic growth factors occurs when oncogenes are activated. These pro-angiogenic growth factors include platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), among others (44).
All these growth factors mobilize endothelial cells and promote their proliferation, making them migrate to the source of angiogenic signals forming blood vessels (18).
VEGF targeted approaches have been proving as the most promising (18). Reasonable success has been achieved when using therapeutics developed to block VEGF pro-angiogenic signaling in the tumor like blocking antibodies (Table 3), soluble decoy receptors and small molecule inhibitors (Table 4) (18).

3.2.1. Blocking Antibodies

3.2.1.1. Anti-VEGF Antibodies: Bevacizumab

Marketed as Avastin, Bevacizumab is a monoclonal anti-body that binds to VEGF and blocks its association with VEGF receptors on endothelial cells. By blocking this

association, it also blocks angiogenesis (18,45).

Table 3. Examples of angiogenic blocking antibodies (18).

Antibody	Target	Indications/Clinical Trials
Bevacizumab	VEGFR-A	First- and second-line metastatic colorectal carcinoma First-line advanced non- squamous non-small cell lung cancer Metastatic renal cell cancer Recurrent glioblastoma multiforme Phase III ovarian cancer FDA approval revoked for metastatic breast cancer
Ramucirumab (IMC-1121b)	VEGFR-2	Phase III: breast cancer, metastatic gastric adenocarcinoma, non-small cell lung cancer, hepatocellular carcinoma, metastatic colorectal carcinoma Phase II: stomach, esophageal, renal, bladder, prostate, ovarian cancers, melanoma and glioblastoma multiforme
CDP-791	VEGFR-2	Phase II: non-squamous non-small-cell lung cancer
IMC-18F1	VEGFR-1	Phase II: breast, colorectal and renal cancers
2C5	VEGFR-3	Phase III: pancreatic cancer Phase Phase I: colorectal cancer

Bevacizumab has three potential mechanisms of action: 1) in monotherapy it has shown an antiangiogenic mechanism effective in the reduction of the tumor growth (46); 2) an anti-hematopoietic progenitor cell mechanism, that is able to inhibit the colonization of the vasculature by circulating endothelial progenitor cells (18); 3) process of vascular normalization. This last one is apparently the main mechanism of action of Bevacizumab.

Normalization is achieved by shaping and redesigning the tumor vasculature to resemble a normal vasculature (18,47). This compound has shown higher effectiveness when used in combination with traditional radio and chemotherapy, because vascular normalization improves blood flow and oxygenation in the tumor, which also improves the delivery of chemotherapeutics to the core of the tumor and turns the tumor cells more radio and chemosensitive (18).

3.2.1.2. Anti-VEGFR-2 Antibodies: DC101, Ramucirumab and CDP-791

VEGFR-2 is one of the main pro-angiogenic VEGF receptor (48). Certain monoclonal antibodies created against the extracellular domain of VEGFR-2 are able to block the binding of VEGF to its receptors and, therefore, inhibit angiogenesis. The first drug of this type that has showed antiangiogenic effectiveness was DC 101 (18). With the help of DC101 studies, it was possible to develop Ramucirumab (IMC-1121B), an anti-VEGFR2 monoclonal antibody, and CDP-791, which is a PEGylated, humanized anti-VEGFR-2F fragment. Studies with Ramucirumab have shown positive results in phase II trials and it is currently being investigated for phase III clinical trials for the treatment of breast metastatic colorectal carcinoma (18,49). A study of CDP-791 in combination with carboplatin and paclitaxel has reached phase II clinical trials in non-small lung cancer. Nevertheless, its development is currently paused as a progression free survival was not improved by CDP-79 (18,50).

3.2.2. Soluble Decoy Receptors

3.2.2.1. Aflibercept

Aflibercept is a recombinant fusion protein composed by the third domain of the VEGFR-2 receptor and the second binding domain of the VEGFR-1 receptor. It acts as a potent competitive inhibitor of VEGFR binding as it binds VEGFR with very high affinity (51). Studies show that Aflibercept is particularly efficient concerning neovascularization originated by macular degeneration, and so, it is a potent angiogenesis inhibitor (18,52). Some trials have shown that Aflibercept in combination with other drugs in the treatment of non-small cell lung cancer enhanced progression free survival but when compared to placebo it did not ensure higher overall survival (53).

Still, in the treatment of metastatic colorectal cancer, Aflibercept improved overall survival relative to placebo when in combination with irinotecan, leucovorin and 5-fluorouracil (54). With that said, Aflibercept is one of the primary drugs used to treat colorectal cancer with numerous positive cases (55).

3.2.2.2. Small Molecule Inhibitors

A different antiangiogenic inhibitor is the Small Molecule Inhibitor, which operates by suppressing downstream signaling from the pro-angiogenic receptors present in the endothelial cells, blocking angiogenesis (18). These types of drugs are inhibitors of the tyrosine kinase receptors such as VEGFRs. This last one is not the only tyrosine kinase receptor that is participating in cancer. As opposed to the previous therapies, this therapy inhibits other mechanisms involved in angiogenesis and tumor induction, and consequently has an anti-tumor activity too. This class of drugs is orally available (18).

Table 4. Angiogenic small-molecule inhibitors (18).

Inhibitor	Activity	Indications/Clinical Trials
Sunitinib	VEGFR-2, PDGFRα and β, c-kit, Flt3, RET	First- and second-line treatment for metastatic renal cell carcinoma Gastrointestinal stromal tumors Progressive, unresectable, neuroendocrine pancreatic tumors Phase III: breast, colorectal and lung
Sorafenib	VEGFR-2 and 3, Raf, PDGFRβ, Flt3 and c-kit	Unresectable hepatocellular carcinoma Advanced renal cell carcinoma Phase III: non-small cell lung carcinoma and melanoma
Pazopanib	VEGFR- 1–3, PDGFRα and β, c-kit	Renal cell carcinoma Soft tissue sarcoma Phase II: non-small cell lung carcinoma, ovarian cancer Phase I: colorectal cancer
Axitinib	VEGFR- 1–3, PDGFRβ, c-kit	Second-line treatment for renal cell carcinoma Phase III: pancreatic cancer Phase II: lung, gastrointestinal, thyroid and breast cancer

Vandetanib	VEGFR- 1–3, PDGFRβ, EGFR, RET	Medullary thyroid cancer Phase III: non-small cell lung carcinoma
Cediranib	VEGFR- 1–3, PDGFRα and b, c-kit	Phase III: recurrent glioblastoma, colorectal cancer Phase II/III: non-small cell lung carcinoma
Vatalanib	VEGFR- 1–3, PDGFRα and b, c-kit	Phase III: colorectal carcinoma Phase II: metastatic neuroendocrine tumors, brain and central nervous system tumors, sarcoma
Brivanib alaninate	VEGFR- 1–3, FGFR1–3	Phase III: colorectal cancer, hepatocellular carcinoma Phase II: renal cell carcinoma, multiple tumor types
BIBF 1120	VEGFR- 1–3, PDGFRα and β, FGFR1–3, Flt3, Src, Fyn, Lck	Phase III: non-small cell lung cancer (NSCLC), ovarian cancer Phase II: breast cancer, prostate cancer, acute myeloid leukemia, glioblastoma, hepatocellular carcinoma, colorectal cancer, high-grade glioma
Tivozanib	VEGFR-2, PDGFR-β, c-Kit	Phase II: NSCLC, colorectal cancer, renal cell carcinoma Phase I: breast cancer, solid tumors

4. Drug carrier delivery systems

Drug delivery systems like nanoparticles take advantage of the pathological and physiological characteristics of tumors to targeted deliver drugs to tumor tissues (17). There are several types of nanocarriers, and each one of them has its singular advantages. Liposomes, polymeric micelles, dendrimers, and polymeric nanoparticles are some of the currently used delivery systems. It is essential that the drug delivery system targets the tumor specifically and selectively. Their mechanism usually operates in three different levels, aiming to adjust the immune system response to enhance treatment, by targeting cancer stem cells to overcome MDR and impede recurrence, blocking the cross talk between the tumor cells and their microenvironment (17). The base of nanocarriers technique relies on the enhanced permeability and retention (EPR) effect, which is a concept that molecules of certain sizes, like nanocarriers, tend to accumulate in tumor tissue much more than they do in healthy tissues duo to the faulty tumor vasculature and the weak tumor lymphatic system (56). Beyond that, the design of nanocarriers has an optimal size and surface characteristics to enhance biodistribution and time circulation alloying it to have controllable drug release capability (17).

4.1. Passive targeting

Taking advantage of the EPR effect, passive targeting relies on the anomalous structure and physiology of the tumor. Drug accumulation in cancer tissues will only occur if the micromolecules avoid the clearance mechanisms such as renal clearance. Nanocarriers efficiency will depend mainly on the capability to remain circulating in the blood stream for a significant amount of time and targeting specific tissues and cells (57).
This capacities are also influenced by the particles shape, size and surface characteristics, and the time circulating in the blood stream is dependent of the interactions with the environment, which can be modified by altering its shape, size and surface characteristics (58).

4.1.1. Size

The size of the particle as an enormous significance on its interaction with the

environment. The determination of the size to be used for drug delivery has to take in account a multitude of factors. In a study published by Dreher and colleagues, its shown that particles in the range of hundreds of nanometers in diameter may accumulate in the tumor tissue. They studied that increasing the molecular weight of a macromolecule as dextran from 3.3 kDA to 2 MDa decreased permeability by two orders of magnitude. When comparing smaller molecules and larger molecules, they observed that smaller molecules could penetrate deeper into the tumor tissue and accomplish a more homogeneous distribution, and the larger molecules were still able to accumulate but were primarily accommodated close to the vascular surface. This can be explained by the fact that the interstitial diffusion coefficient decreases as the molecular weight of the diffusing particle increases (59). Nanoparticle that are able to participate in the EPR effect have the maximum size of approximately 400 nm (60). Particles that are bigger than 400 nm cannot diffuse through the tumor tissue in quantities needed to have a clinical or therapeutic effect. There are still other important aspects that we have to take in account when choosing an effective nanoparticle size range. The faulty vasculature of tumors is in great part due to the boost of size and quantity of fenestrations, which are commonly 50-100 nm in size. When we look into clearing mechanisms, particles with a diameter bigger than 200 nm will be cleared much faster than particles with less than 200 nm (60,61). Considering all the variants, it has been determined that the maximum effective particle size is 150 nm (62). To conclude, a nanoparticle should have between 10nm and 150 nm to assure increased accumulation in the tumor tissue and longer circulation time (61).

4.1.2. Particle shape

In a study by Chan *et al*. it has been shown the effects of particle shape and curvature on cellular internalization. Spherical nanoparticles between 14 and 75 nm were absorbed 3.75-5 times more than 14 by 74 nm rod shaped particles (61,63).

This difference in absorption, can theoretically be consequence of the curvature which affects the contact area with the cell membranes, and also the distribution of targeting ligands on the surface of the particles (61).

In a different study, it is reported that the cylindrical nanoparticles were the most efficient, specifically nanoparticles with a diameter of 150 nm and height of 450 nm. This cylindrical cell was taken 4 times faster than symmetrical particles with an aspect ratio of

1, which suggests that the aspect ratio is another important variant in cellular uptake. Particles with 100nm diameter and an aspect ratio of 3 were less absorbed than particles with the same aspect ratio and 150 nm diameter. This could mean that the absorption mechanism is depended on a function of size and shape.

4.1.3. Surface Characteristics

The main mechanism by which a particle interacts with its environment is through its surface. Since nanoparticles have a relatively large surface and a large surface to volume ratio, it is very important to study its surface characteristics (64).
It is possible to modify the surface through polymer content and functionalization, which will change the way the particle is perceived by the environment (61).
It has been studied that adding hydrophilic polymers to the polymer structure can decrease clearance by the MPS/RES system (64). When polyethylene glycol (PEG) was used attached to the surface of the particles, it shielded the nanoparticles from opsonin adsorption and consequently clearance by the MPS/RES (60).
In has been proven that increasing molecular weight of PEG chains, increments the half-life of nanoparticles. It is possible to modify the length, shape and density of PEG chains, and each modification has a different effect on the clearance rate (65).

4.1.4. Limitations of Passive Targeting

Passive targeting consists on achieving the perfect size, shape and surface characteristics of the drug carrier. Nevertheless, there are still some obstacles such as insufficient drug concentrations in the tumor tissue which will end up in a very little therapeutic effect (66). Additionally, this kind of targeting still suffers from some of the traditional chemotherapy limitations, since it is unable to perfectly distinguish tumor tissue from healthy tissue (61,67).

4.2. Examples of delivery systems in cancer

4.2.1. Liposomes

Liposomes are self-assembled colloidal particles which are composed by both polar and nonpolar amphiphilic molecules. When it is put together, it creates a spherical structure

where the polar elements interact with the polar surroundings, and the nonpolar elements act the same way with the nonpolar environment (61,68).

Liposomes are since its beginning a very alluring drug delivery system as its composition and structure is very much alike to cell membranes. Furthermore, natural, nontoxic, non-immunogenic biodegradable amphiphilic molecules can be used to quickly form liposomes (69). Liposomes are commonly classified by its total lipid bilayers composing the colloidal structure. Multilamellar liposomes have numerous lipid layers, while unilamellar liposomes are composed by only one lipid bilayer (68).

These kinds of structures when acting alone lean to have a quick clearance from the bloodstream due to its light sterically instability. However it can be corrected by using polyethylene glycol to functionalize the liposome surface and consequently give an enhanced steric stabilization (68).

Additionally, liposomes can be used in active targeting by altering the liposomal surface with some ligands. The first liposome-based cancer treatment used a PEGylated liposome that had doxorubicin encapsulated (69).

4.2.2. Micelles

Micelles are structures that present a hydrophilic exterior and a hydrophobic nucleus, made of amphiphilic molecules that have the capability to self-assemble into this structure (70). Micelles have numerous advantages in the drug delivery systems as they usually have a diameter of less than 100 nm, which allows them to engage through the fenestrations in cancer vessels and limit the uptake by the MPS/RES system. Hydrophobic drugs are encapsulated into the center of the micellar structure and protected by the hydrophilic , and are carried to the cancer tissue (71). The hydrophobic exterior also protects the micelle from quick recognition thus enhancing the circulation time (72).

4.2.3. Dendrimers

This type molecule has a very well-defined structure which is extremely branched and presents a large degree of monodispersity (73).

It is widely used due to its surface which can be quickly functionalized, and it can also be incorporated with targeting ligands and molecules as folic acid. The dendrimers multifunctional core has the capability to encapsulate and protect drug molecules by its

large branching. Furthermore, some drugs can also be incorporated to the exterior of the dendrimer (74).

4.2.4. Nanocapsules and Nanospheres

Nanocapsules carry the drug molecules in its core separated from the surroundings by a polymeric membrane (69). The main characteristic that allows it to be used in drug delivery systems is the fact that the drug can diffuse through the membrane in a constant pace due to the core saturation in active substance.

In nanosphere technique, it is used a polymeric matrix that encapsulates the drug. Usually, the active substance is evenly distributed in the matrix and can be released by diffusion. The amount of time prior to the drug release is dependent on the composition of the polymer matrix and also by its ability to absorb fluids (67).

These nanoparticles high surface to volume ratio is one of the main reasons why it is such a used method, as its large surface area allows drug time scales release to be viable and clinically relevant. There has been great concern in the development of systems that do not only rely in the diffusion mechanisms. New particles have been studied to respond to chemical, thermal, environmental and biological sparks (70,75). The main objective of these new particles is that they can actively release its active substance only when they are triggered (61).

4.3. Active targeting

Active targeting is a system which uses molecular recognition such as antigen-antibody and ligand-receptor to specifically delivery a drug (69).

Active targeting takes an important role in cancer therapy because it has the particularity of eliminating or reducing the delivery of toxic drugs to healthy tissue. Therefore targeted delivery of nanoparticles can increase therapeutic effectiveness and reduce side effects (66). Some tumor cell surface receptors such as transferrin and folate are overexpressed, and active targeting takes advantage of that (62). The most used active targeting ligands in cancer therapy include, transferrin, folate, aptamers and monoclonal antibodies since this type of nanodelivery have performed considerably better than non-targeted mechanisms and showed reduction of healthy tissue damage and higher cytotoxicity to cancer cells (76).

4.3.1. Transferrin

Transferrin is a receptor-ligand pair that works with its receptor TfR. It is a membrane glycoprotein which takes part on the iron uptake cell mechanism. Basically, endocytosis occurs when transferrin binds to its receptor, and it is internalized into the cellular cytoplasm (77). Transferrin receptors are an attractive option when it comes to targeted delivery via nanoparticle carriers because it is overexpressed by 10-fold on cancer cells (78).

Two materials commonly used in drug delivery, poly(D,L-lactic-co-glycolic acid) (PLGA) and polyvinyl alcohol (PVA), were used by Sahoo and colleagues in the development of transferrin conjugated paclitaxel loaded nanoparticles, where transferrin was attached to the nanoparticle surface and loaded with paclitaxel. They used them in human prostate cancer treatment and compared them with non-transferrin nanoparticles. When compared, the transferrin nanoparticles presented a continuous release profile and a three times more cellular absorption. Additionally, the transferrin conjugated nanoparticles reduced tumor cell proliferation by 70%, against only 35% in non-conjugated nanoparticles. (61,78). Nanoparticles conjugated with transferrin have shown good results in inhibiting tumor growth and cell proliferation, because it has a sustained release profile and increased cellular absorption. Its effectiveness can theoretically be explained by its capability to be absorbed by receptor mediated endocytosis, which limits the amount of drug delivered to healthy cells, and increased the amount delivered to cancer cells.

4.3.2. Folic acid and folate

Folate is also one of the most used ligands for targeted drug delivery devices. Similarly to transferrin, it induces endocytosis when it binds to its receptor (FR), which binds with a high affinity (61,66,79). Many tumors such as the meningiomas, osteosarcomas, ovarian carcinoma and non-Hodgkin's lymphomas express this receptor (80). When added to nanoparticles, the folic acid or folate binds, crosses into the cytoplasm, the drug is released and interacts with intracellular components (69).

Yoo and colleagues used a biodegradable polymeric micelle loaded with doxorubicin conjugated with folate. They created micelles from poly(D,L-lactic-co-glycolic acid) and PEG. This last one is used to enhance the circulation time of the nanoparticles. PLGA

allows the nanoparticle to biodegrade after delivering the drug. The folate was chemically conjugated to the PEG and the doxorubicin was added to the PLGA. Doxorubicin can present cardiotoxicity and cytotoxicity, and because of that, the nanoparticles were compared to non-targeted doxorubicin on folate receptor cell lines. It was determined that these new nanoparticles shown increased circulation time, enhanced cellular absorption and decreased cardiotoxicity (81) . This decrement suggests that this drug delivery method is able to make a distinction between tumor and healthy tissues with much more specificity than non-targeted doxorubicin (61,81).

4.3.3. Aptamers

Aptamers are short oligonucleotides of RNA or DNA that are able to fold into a range of conformations and participate in ligand binding (66). It is very difficult to find this type of sequences, since only one in each 1010 random RNA sequences can engage in ligand binding. However, there is a process called, systematic evolution of ligands by exponential amplification (SELEX) that enables researchers to examine through vast RNA and DNA sequences to find aptamers (82). Aptamers studies, similarly, to folate and transferrin, have shown increased targeting specificity and an efficient drug delivery system to tumor cells. Additionally, its benefits include lack of immunogenicity and ability to immediately penetrate and target tumor cells (66).

For the treatment of prostate cancer, it has been created an aptamer-conjugated nanoparticle. The objective of this nanoparticle was to target specifically a prostate specific membrane antigen (PSMA) that is overexpressed in prostate tumor cells.

The drug used was cisplatin which functions by interfering with DNA transcription, and when administered systemically is usually ineffective against prostate tumor cells.

PLGA and PEG was used to encapsulate the cisplatin. When compared to free cisplatin, the PSMA aptamer nanoparticle was 80 times more harmful to tumor cells expressing PSMA (83).

4.3.4. Antibodies (Monoclonal Antibodies)

Such like the aptamers, it is possible to target specific antigens present on cell membranes by attaching antibodies into the surface of nanoparticles. In the past years, the use of antibodies has been exhaustively investigated and therefore, there is a great variety of

available treatments (84,85). Unconjugated antibodies have shown to have effective therapeutic qualities on colorectal cancers, lymphomas and chronic lymphocytic leukemias (85).

Treatments involving antibodies function by interaction with specific antigens present on the surface of the tumor cell. Once the interactions occurs, it has multiple antitumor mechanisms such as suppression of protein expression and interfering with ligand-receptor binding(61,85). In the beginning of the antibody-based targeting, there was a considerable amount of limitations, since the antibodies were often derived from mice and in some cases culminated in an immune response that limited the effectiveness of the treatment. The lack of specificity and capable targeting to their antigen-binding sites was a limitation too (86).

Nowadays, with more recent technology, antibodies originate from murine proteins, which can be modified into humanized forms that induce almost no immune response. The lack of specificity is now surpassed by molecularly modifying binding regions to target a vast diversity of receptors. One example is the IgG molecule which contains a biding region able to identify antigens and can easily be manipulated to specifically recognize a range of targets (86).

One of these targets is the epidermal growth factor receptor (EGFR). This receptor is overexpressed in a variety of cancers and binds to two ligands, transforming growth factor -alpha and epidermal growth factor (87). When either one of these ligands binds to the EGFR, the growth of cancer cells is stimulated. This ligand-receptor interaction is responsible for the fast cancer cell proliferation.

It is possible to reduce or stop the proliferative behavior of cancer cells by blocking the binding via antibody interference (87).

In a study by Hoffman and his colleagues was observed an increase of the cytotoxic effect in some cancers by molecules combining anti-EGFR antibodies with doxorubicin and cisplatin, and even in some cases totally eradicating the tumor (88).

In a different experience, two distinct biodegradable polylactic acid (PLA) nanoparticle formulations were tested, using rituximab mAb (CD20 antigen) and trastuzumab mAB (HER2 antigen). They observed that this molecules bonded to their respective antigen 10 times more than on non-targeted nanoparticles (89).

Monoclonal antibodies have been proving being one very good targeting technique due to its specificity that has the capability to actively target and differentiate between healthy and tumor cells as much as tumor cell types (61).

In a study by Johnston and colleagues, based on the fact that in 95% of colorectal cancers there is an expression of the A33 antigen, they used huA33 mAB (A33 monoclonal antibody) to specifically target colorectal tumor cells expressing the antigen (90).
They design a polymeric nanoparticle system made of a silica core followed by a layer-by-layer deposition of alkyne-modified poly(N-vinylpyrrolidone) and poly(methacrylic acid), which was then conjugated with the A33 monoclonal antibody. The team observed that the particles interacted preferentially with tumor cells and were phagocytosed, which is excellent for the delivery of chemotherapeutic drugs (61,90).
Even though the antibody technique has been showing good results, there are still many limitations that need to be tackled. The fact that the manufacturing and manipulation of antibodies is a complicated and costly mechanism that is hard to upscale to a larger scale (86). Cancer tissue penetration can sometimes be non-uniform, and there is still a potential immune response even with entirely humanized antibodies (91).
Since the lack of tumor penetration is caused by increased size of nanoparticles due to antibodies size, antibody fragments have been used as an alternative as they are minor and induce less immune response , while still being able to target antigen receptors on the surface of the cancer cells (61,66,91).

4.3.5. Limitations of Active Targeting

Active targeting mechanisms are able to improve the bioavailability of delivered drugs and reducing off-target effects. Nevertheless, there is a diversity of limitations that still need to be outpaced (61).
Although active targeting ligands aim to increase and reinforce nanoparticle accumulation in cancer tissue, it is not absolutely known if this higher concentration of carriers have any influence in the delivery of the drug into the interior of the cancer cell. In other words, admitting that nanoparticle carriers are able to accumulate in cancer cells, their efficacy is determined by their capability to deliver the drug (76). The receptor mediated endocytosis comes with the issue of endosomal escape once the carrier is hooked (92). Furthermore, the substitution of polymers with active targeting fractions can negatively affect the clearance and opsonization of the carrier. Active targeting ligands are only effective if they find cancer cells expressing compatible agents. If there is a fast clearance, there will be an accumulation of carriers in RES organs and the cancer tissue will accumulate a fewer amount of targeted nanoparticles (76). In one hand, active

targeting ligands surpassed a great number of passive targeting obstacles, but in other hand, there is still much work needed to increase biodistribution and efficacy of this nanoparticles (61).

5. New Strategies

5.1 Doxorubicin-Loaded Liposomes

Deshpande *et al.* started by demonstrating a study where by adding active tumor-targeting arginine-rich cell penetrating peptides (AR-CPP) to the surface of the Doxorubicin loaded liposome could enhance drug delivery both to nuclei and cytoplasm, while also showing greater effectiveness therapeutically (93). Later, in a different study, the authors added transferrin and AR-CPP octaargine (R8) to the doxorubicin liposomes surface with the objective of better target the A2780 ovarian cancer cells. This molecule is called Dual DOX-L. This system relies on the over expression of transferrin receptors and also in the doxorubicin delivery which will be mediated by the R8 (93). It showed a two times higher tumor-cell association when compared to other treatments after 4 hours of treatment. Furthermore, Dual DOX-L showed an improved cytotoxicity *in vitro* and a more efficient tumor growth control when compared to other therapies. Doxorubicin accumulation in cancer tissue was also higher after the treatment with Dual DOX-L. Concluding, all the results have shown that the association of transferrin and R8 takes a good advantage of the transferrin receptors over-expression , enhancing specificity and also of the R8 mediated intracellular delivery, and that his association clearly results in a more effective therapeutic potential (93).

5.2 Paclitaxel-Loaded Perlecan Targeted Nanoparticles

Khana *et al.* identified that a certain cell surface protein called perlecan was overexpressed in breast cancer tumor cells (94). Perlecan is essential for normal growth as it is highly glycosylated and has an important job linking growth factors.

Other than its own receptors, growth factors such as VEGF-A and fibroblast growth factor receptor FGF-2 also bind to perlecan, making perlecan a good target for drug delivery. In their study, they also proved that the breast cancer patients with a greater perlecan

expression also had a decreased survival rate when compared to the patients with low perlecan expression, which could aim to a clinical significance of this target. With the objective of studying the effectiveness of this target, they developed a monoclonal antibody (AM6) that has a high affinity to tumor cells that express the perlecan protein. Using PLGA as a nanocapsule, paclitaxel was encapsulated as a chemotherapeutic agent and later on AM6 was added to its surface. This nanoparticle therapeutic function was studied using *in vitro* models of breast cancer cells. The paclitaxel loaded nanocapsule shown enhanced cancer growth blockage, achieving 44% of tumor inhibition in the end of the treatment (94).

5.3. Parvifloron D-Loaded Albumin Nanoparticles

In a very recent study by Santos-Rebelo *et al.*, the low water solubility of Parvifloron D was surpassed using nanotechnology. Parvifloron D is a natural origin compound extracted from Plectranthus genus that has shown cytotoxic effects in cancer cells and also the ability to reduce the proliferation of the cells (95). In this study, bovine serum albumin was used to encapsulate the drug since it is one of the most abundant protein in the blood plasma. Besides, it is biodegradable, biocompatible and nontoxic (96). It also shows a specific and active targeting in the liver-pancreas system (95,97). Being the target pancreatic cancer cells, cetuximab and erlotinib which are EGFR inhibitors were attached to the bovine serum albumin nanoparticles surface. These EGFR receptors are generally overexpressed in pancreatic cancer (98). This association of the two antibodies demonstrated a huge improvement in the antiproliferative effect, being that the IC50 was more than 4 times less when Parvifloron D was conjugated cetuximab and erlotinib albumin nanoparticles than in non-conjugated particles (95).

5.4. Subcellular targeting

Other than targeting specific cells, it is also possible to target specific organelles inside a cell. In a study made by Han *et al.* a system was developed to target the nuclei of cancer cells using pH responsive core-shell structured nanoparticles (CSNPs) (99). These CSNPs had a triple stage targeted delivery of doxorubicin. The external layer was made of an amino functionalized mesoporous silica nanoparticle assembled with TAT peptide and acid-cleavable PEG. This layer decreased the clearance of CSNPs in the blood flow which

facilitated the passive accumulation in the tumor tissues. Via acidic hydrolysis, the PEG layer detaches and exposes the anionic shell made by galactose-modified poly(allylamine hydrochloride)-citraconic anhydride, which is a hepatocarcinoma targeting molecule. This layer was the responsible for active internalization of the nanoparticle into the cancerous cells. After the internalization, the acidity of the lysosomes and endosomes activates the conversion of the anionic layer which leads to disassembly and consequently TAT mediated delivery of the doxorubicin to the nuclei. As a result, researchers claim an enhanced tumor distribution of the CSNPs and an impressive therapeutic effect with a relatively low dose. This analysis suggests that the CSNPs may be a powerful, efficient and nontoxic system for the targeted delivery of anticancer drugs (99).

6. Conclusion and future perspectives

Cancer is still one of the leading causes of death in the whole world. Every day, innumerous efforts are being made aiming to find the best therapy to treat this disease. Conventional therapies such as surgery, radiotherapy and chemotherapy lack in eradicating the tumor in specific manner. Thus, in most cases, those therapies are not able to provide a comfortable lifestyle for cancer patients.

The discovery of target delivery drug systems is an enormous breakthrough in cancer therapy as it focuses on actively targeting tumor cells, providing a better delivery with the adequate load of drug.

Many strategies were studied and herein described, discovering new ways to target specific overexpressed receptors in cancer cells as can be seen in Table 5. Although many progresses have been made, there are still many obstacles. One of those challenges is the fragmentation between the research and pharma market coupled to an academia-industry interlinking that needs to be improved. A novel paradigm with a suitable alignment between technological developments and the patient needs through a jointly and multidisciplinary approach is crucial. On the other hand, it is known the current gap of specific guidelines for these new nanomedicines. It is known that several efforts are being made to strongly prompting the emergence of scientific-technological networking initiatives assembling international experts for guidance for industry to produce those products but overall, the future seems optimist and will bring for sure innovative and successful treatments for cancer.

Table 5. Resume of MTD, blocking antibodies and drug delivery systems

Micro Tubule- Destabilizing Agents	
Combretastatin A-4 disodium phosphate (CA4P)	CA4P binds to tubulin avoiding its polymerization as it is cleaved by endogenous phosphatase, caused large vascular damage inducing hemorrhagic necrosis and consequent cancer growth delay
Combretastatin-A1	Combretastatin derivative binds to tubulin avoiding its polymerization as it is cleaved by endogenous phosphatase, caused large vascular damage inducing hemorrhagic necrosis and consequent cancer growth delay

Ombrabulin	Combretastatin derivative which has shown ability to cause large cancer core necrosis by quickly shutting down the cancer blood flow
ADH-1	Inhibits the N-cadherin binding site and consequently decrease blood supply, causing hemorrhage necrosis in animal cancer models
Blocking antibodies	
Aflibercept	Acts as a potent competitive inhibitor of VEGFR binding as it binds VEGFR with very high affinity
Bevacizumab	Monoclonal anti-body that binds to VEGF and blocks its association with VEGF receptors. By blocking this association, it also blocks angiogenesis.
Ramucirumab (IMC-1121B)	Is an anti-VEGFR2 monoclonal antibody able to block the binding of VEGF to its receptors and, therefore, inhibit angiogenesis
Drug delivery systems	
Transferrin	PLGA and PVA nanoparticle conjugated with transferrin and loaded with paclitaxel
Folate	PLGA and PEG micelle loaded with doxorubicin conjugated with folate.
Aptamers	PLGA and PEG aptamer conjugated nanoparticle loaded with cisplatin

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